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Research Progress in In-situ Gelling Ophthalmic Drug Delivery System

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ABSTRACT

Blindness and vision impairment are the most devastating global health problems resulting in a substantial economic and social burden. Delivery of drug to particular parts of the anterior or posterior segment has been a major challenge due to various protective barriers and elimination mechanisms associated with the unique anatomical and physiological nature of the ocular system. Drug administration to the eye by conventional delivery systems results in poor ocular bioavailability (< 5%). The designing of a novel approach for a safe, simple, and effective ocular drug delivery is a major concern and requires innovative strategies to combat the problem. Over the past decades, several novel approaches involving different strategies have been developed to improve the ocular delivery system. Among these, the ophthalmic in-situ gel has attained a great attention over the past few years. This review discussed and summarized the recent and the promising research progress of in-situ gelling in ocular drug delivery system.

Key words: In-situ gel; Ocular; Drug delivery; Bioavailability; Polymer; Corneal retention

1. Introduction

The eye is a complex and unique part of the human organs that has been considered as the window to the human soul. Broadly, the human eye is divided into two segments that are anterior and posterior segments (Fig. 1) [1]. The specific disease conditions of the eye are associated with each of these broad segments. For instance, conjunctivitis, glaucoma, blepharitis, and cataract are some of the diseases that affect the anterior segment of the eye, while diabetic retinopathy and age-related macular degeneration are known to affect the posterior segment [2].

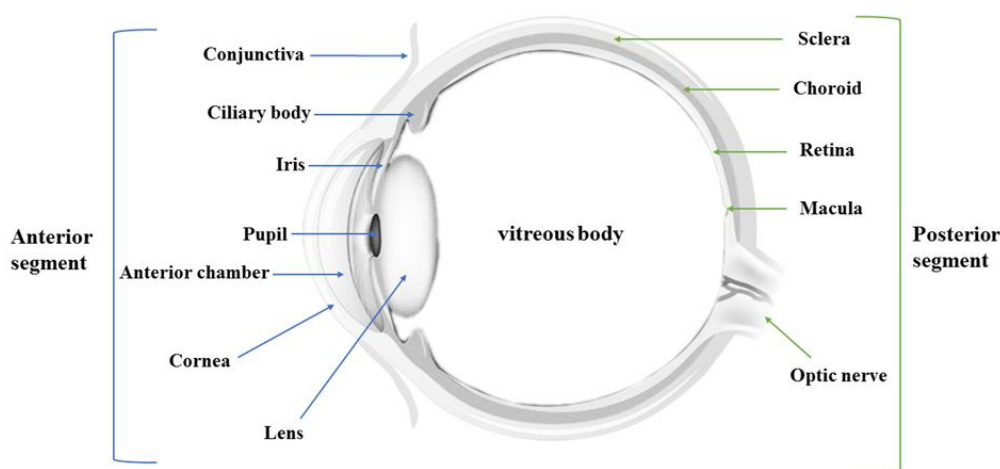


Fig.1-The anatomy of ocular system: the anterior segment involves conjunctiva, ciliary body, iris, pupil, anterior chamber, cornea and lens; the posterior segment consists of sclera, choroid, retina, macula and optic nerve.

Due to the unique structure of the eye, which inhibits the entry of drug molecules into the desired site, the ophthalmic delivery of the drug has been one of the most challenging tasks for a pharmaceutical scientist. Eye drops accounts for more than 90% of ophthalmic preparations on the markets. However, they are washed away from the eye and results in low ocular bioavailability (< 5%) after topical administration [3] by different elimination mechanisms. This elimination process includes tear turnover, nasolacrimal drainage, protein binding, systemic absorption, enzymatic degradation and complex penetration barriers (Corneal Barrier, Blood Aqueous Barrier (BAB), and Blood Retinal Barrier (BRB)) [4] (Fig. 2).

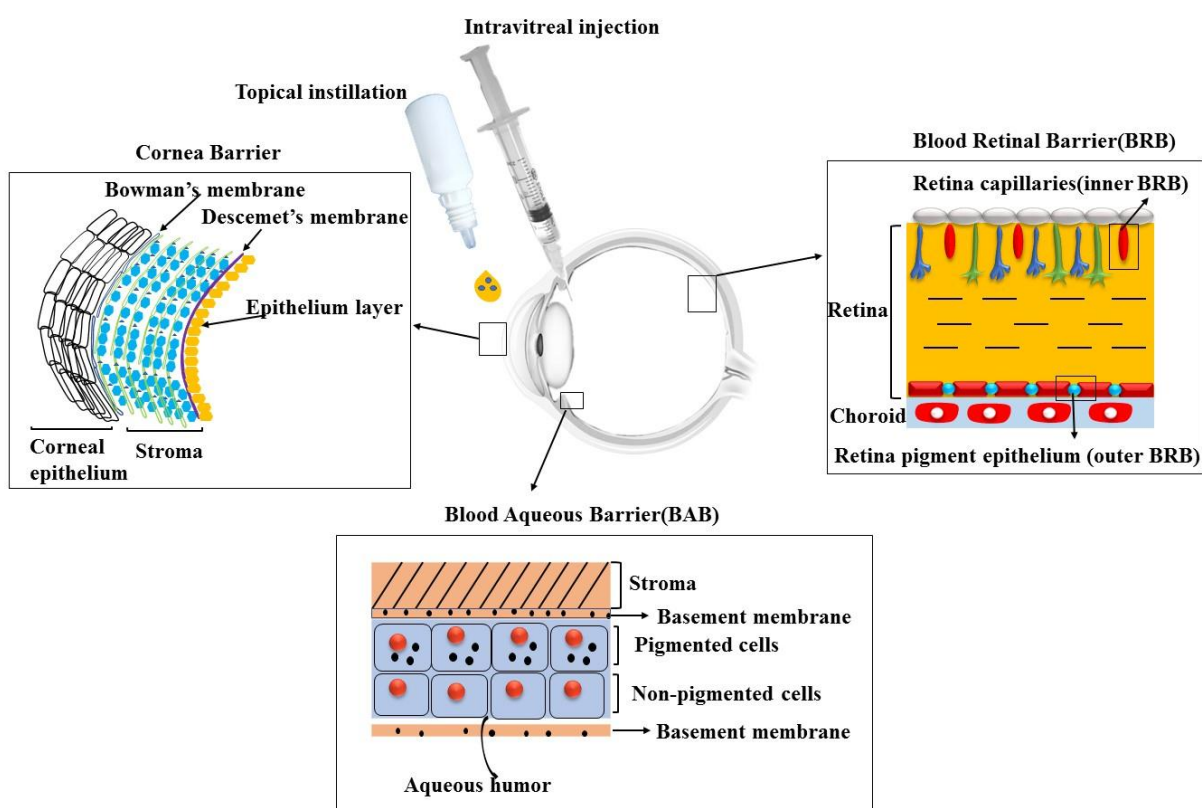


Fig.2-The critical barriers to ocular drug delivery systems: the Corneal Barrier: involves of epithelial layers attached together by tight junctions avoiding entry of drug particle followed by thick stroma and endothelial cells. The Blood Retinal Barrier (BRB): comprises of the inner BRB resulted from retinal capillaries. Blood Aqueous Barrier (BAB): made by the nonpigmented cells of the epithelium of the ciliary body, and the endothelium of the iris blood vessels.

One of the main drawbacks in ocular drug delivery is achieving and retaining of optimal concentration of drug at the desired site of action in the eye. Several ophthalmic dosage forms such as ointments, eye drop solutions, gels, and ocular inserts have been investigated in order to prolong the ocular residence time of drugs after the topical application to the eye. With these formulations, the corneal contact time has been increased to some extent. But, due to blurred vision and poor patient compliance resulted from ointments and inserts, respectively, they have not been fully accepted [5]. Furthermore, drugs that are administered systemically to exert their action in the ophthalmic system also have known to access poorly to the eye tissue [6]. Intravitreal and peritocular routes are recommended in order to deliver drugs to the posterior part of the eye. However, there are disadvantages associated with these routes like the frequent

intravitreal injections could be painful, thus affecting a patient compliance. The periocular route is easy for administration, but the static and dynamic barriers constitute a problem [7].

The low bioavailability of medications from the conventional delivery system is resulted from a great extent of precorneal drug loss by nasolachrymal drainage. The rapid clearance of the topically applied drug into the eye often results in a short duration of pharmacological activity and, therefore, the need for a frequent dosing regimen. Moreover, 50%-100% of an instilled dose could undergo systemic absorption through drainage via the nasolachrymal duct. This could lead to a possible increased risk of unwanted systemic toxic effects [8].

In last decades, various delivery systems such as using chemical permeability enhancers [6], prodrugs [9] stimuli-responsive in-situ gel [10], and drug delivery carriers such as liposomes [11] and nano- or microparticles [12], noisomes [13], dendrimers and microneedles [14] have been developed to increase ocular residence time, drug penetration across the ocular barriers and ophthalmic bioavailability. In-situ gelling system is one of the promising approaches to improve the retention time of drugs on the ocular surface. After instillation of the aqueous solution containing stimuli-responsive polymers such as pH-sensitive polymers, thermosensitive polymers, and ion-sensitive polymers, the viscous and mucoadhesive gels are formed on the eye surface [15], subsequently, ocular retention time and ocular bioavailability of the ophthalmic drugs are improved. Therefore, in this review, we summarized and discussed the most recent research innovations in stimuli-responsive in-situ gelling system for ocular drug delivery system.

2. Anatomy of the ocular system

Generally, the eye is divided into two important segments: (1) The anterior segment which involves the cornea, conjunctiva, iris, pupil, ciliary body, anterior chamber, aqueous humor, lens and trabecular meshwork. (2) The posterior segment includes vitreous humor, sclera, retina, choroid, macula and optic nerve (Fig. 1) [1].

The cornea is the transparent and clear avascular part of the ocular system that forms the anterior most coat of the eye. Anatomically, the cornea is consist of five major layers. Corneal epithelium is the first layer, which is the most exterior [16]. The other layers include Bowman's membrane, stroma, Descemet's membrane and the endothelium layer [1]. Corneal permeability is the most essential factor that determines drug concentration in aqueous humor. For most of hydrophilic

drugs, the epithelium is a rate-limiting barrier of transcorneal diffusion of drugs [17]. The stroma is owing to the hydrophilic nature, it acts as a barrier for the diffusion of highly lipophilic drugs [17]. The corneal stroma is mainly consisting of charged and highly organized hydrophilic collagen that inhibit the diffusion of hydrophobic molecules [18].

Conjunctiva is a clear thin membrane that covers the sclera and lines the inner surface of the eyelid. It is consist of stratified epithelium (non-keratinized) and goblet cells. It provides protection to the eyes by secreting mucus that prevents entry of microorganisms and lubricating the eyes [1]. In humans, the conjunctiva occupies a 17-times larger surface area than the cornea. This allows for greater absorption of the drug to occur through the conjunctiva. Therefore, drugs are usually more permeable across the conjunctiva than the cornea. However, absorption of the drug via the conjunctiva is still not significant due to the existence of conjunctival blood capillaries and lymphatics, which leads to a considerable loss of drug into the systemic circulation, thereby reducing the overall ocular bioavailability [19].

Aqueous humor consists of clear liquid that fills both the posterior and anterior chambers of the eye. The aqueous humor is non-vascular structure that must be transparent to allow light transmission, which provides nutrition for the cornea [1]. It contains excessive concentration of ascorbate which is about 15-fold the concentration in the plasma, and has a pH of 7.2 [16]. Its main function is to provide nutrients, eliminate waste from non-vascular tissues and control the intraocular pressure that keeps the convex shape of the cornea [20].

The sclera is the whitish portion of the eye, opaque and elastic in nature, consisting of collagen fibers [1]. The solutes especially hydrophilic compounds are generally more permeable across the sclera than the cornea and the conjunctiva, because diffusion through sclera is mainly a matter of transport across an aqueous medium of proteoglycans or leaky spaces within the collagen network rather than diffusion across cellular membranes [19]. The sclera offers a protective outer layer, maintaining intraocular pressure and serving as the attachment portion for the extraocular muscles [17].

The retina is a multiple layers and complex structure that consists of vascular, glial and neural, cells and nerve fibers [16]. The retina is a major barrier to ocular delivery of drug with larger molecular weight [19].

3. In-situ gelling system

Ophthalmic in-situ gelling is comprising of environmentally sensitive polymers that will be altered structurally with the small changes in specific conditions like pH, temperature and ionic strength in the environment. In-situ forming gels are liquids during instillation into the eye and then undergoes rapid gelation in the cul-de-sac of the eye to form viscoelastic gels in response to environmental changes (Fig. 3); lastly release the drug slowly under physiological conditions [21]. Consequently, the residence time of the gel formed in-situ will be extended and the drug is released in a sustained manner which leads to enhanced bioavailability, minimized systemic absorption and reduced frequent dosing regimen resulting to improved patient compliance [22]. Furthermore, some other potential advantages such as simple manufacturing process, ease of administration, and deliverance of accurate dose have been exhibited by in-situ gelling systems [23].

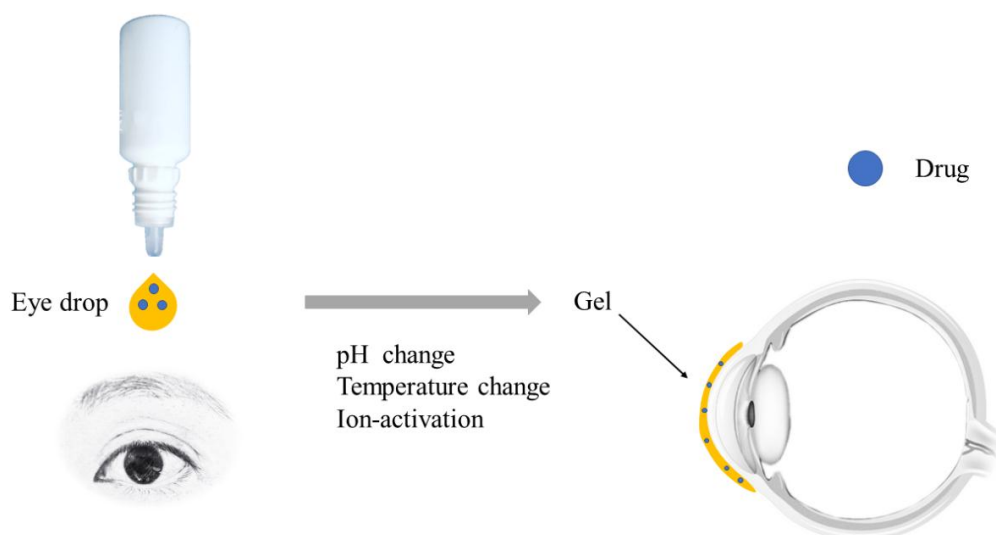


Fig. 3-In-situ forming gels process. The formulation is liquid when instilled into the eye which undergoes gel formation rapidly in the cul-de-sac of the eye in response to environmental changes such as pH, temperature and ion; finally release the drug slowly under physiological conditions.

3.1 Mechanisms of gelling system

In-situ gel formation may be achieved by a number of mechanisms including temperature- (Fig. 4), pH- and ion-activated systems. Temperature triggered in-situ gel system which utilizes the temperature sensitive polymers that exist as a liquid form below its low critical solution temperature (LCST) and undergoes gelation when the environmental temperature reaches or is

above the LCST [24]. The pH induced in-situ gel contains polymers which possess acidic or alkaline functional groups within the chain molecule and undergoes a sol-gel phase transition on change from a low pH to high pH environment [23]. Ion-activated systems are also known as osmotically triggered in-situ gel systems wherein the polymer undergoes a sol-gel transition due to changes of ionic concentration, which is typically triggered by mono or divalent cations in tear fluid particularly Na^+ , Mg^{2+} and Ca^{2+} [25]. In addition, sol-gel phase transition has known to be induced by enzymatic cross linking and photon polymerization [25, 26]. However, the pH, temperature, and ion-induced in-situ gel are the most extensively studied approaches of in-situ gel, and the concern of this review.

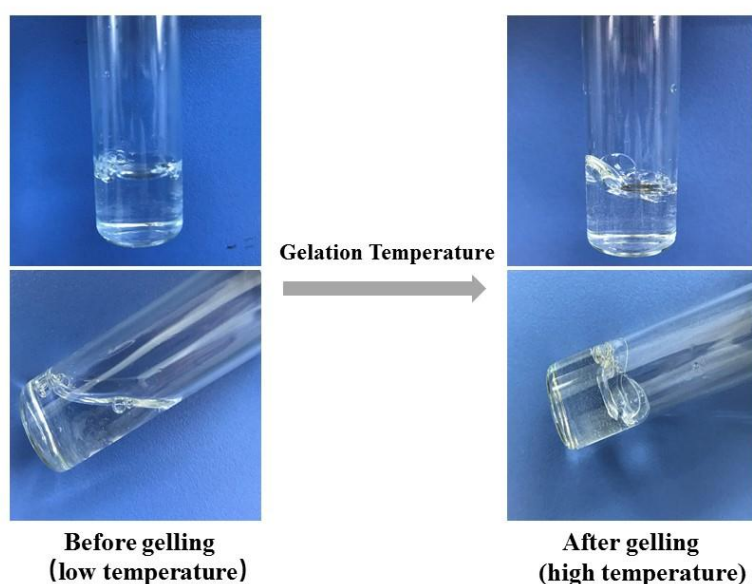


Fig. 4-The gelation process of thermosensitive in-situ gelling. When the temperature is below the gelation temperature (GT), it's clear solution with low viscosity, upon heating it to GT, the solution is converted to the gel with high viscosity.

3.2 Stimuli-responsive in-situ gel system

3.2.1 Temperature-triggered in-situ gel systems

The temperature sensitive in-situ gel is the oldest, the most extensively studied and common type of stimuli-responsive gel. It can be easily and precisely introduced into the eye in liquid form without producing irritation or blurred vision. The gel is formed at the precorneal temperature (35 °C) to endure the lachrymal fluid dilution without rapid precorneal elimination of instilled

drug after administration [27]. It has been recommended that a good thermo-responsive ocular in-situ gel should possess the gelation temperature above the room temperature and undergo gel-sol transition at a pre-corneal temperature in order to avoid storing in a refrigerator before instillation, which may sometimes result in eye irritation due to cold nature [28].

3.2.1.1 Polymers used in temperature triggered in-situ gel systems

Poloxamers (Pluronic)

Poloxamers are a triblock copolymer poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) (PEO-PPO-PEO) exhibiting amphiphilic nature because of hydrophilic ethylene oxide domains and hydrophobic propylene oxide domains [29] (Fig. 5). The triple block of copolymers PEO-PPO-PEO (Pluronic or Poloxamers) undergo gelation at body temperature in concentrations above 15% (w/w) [30]. The principal possible mechanisms have been proposed to explain the sol-gel phase transition at an increased temperature are the gradual desolvation of the polymer, enhanced micellar aggregation, and the increased entanglement of the polymeric network [26]. The pluronic triblock copolymers are existing on the market in different grades with different physical forms and molecular weights. Depending upon the physical description for the grades are given as L for liquid, P for paste and F for flakes. The commonly used poloxamers are 188 (F-68), 237 (F-87), 338 (F-108) and 407 (F-127) [31]. Pluronic F-127 (F-127) or Poloxamer 407 (P407) (copolymer PEO106-PPO70-PEO106) consists of ethylene oxide (70%) which contributes to its hydrophilic property. F-127 is a copolymer with molecular weight of 12 000 Da, a PEO/PPO ratio of 2:1, nontoxic, with low viscosity below 4 \square and forming a semisolid gel at body temperature. Furthermore, F-127 has better solubility in cold water than in hot water because of the hydrogen linkages at low temperatures [31, 32].

Xyloglucan

Xyloglucan is a polysaccharide obtained from tamarind seeds, therefore it is often named tamarind seed polysaccharide (TSP), which when partially degraded by β -galactosidase displays thermally reversible gel formation in diluted aqueous solution (Fig. 5). The sol-gel transition temperature is varying with the degree of galactose degradation [33]. TSP gels have been reported to have a potential for oral, ocular, intraperitoneal and rectal drug delivery [23, 33]. TSP is highly water-soluble and gelation occurs when the galactose elimination exceeds 35% [34].

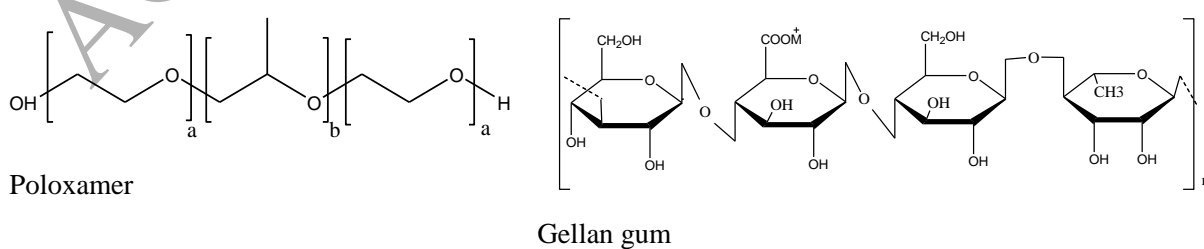
Cellulose derivatives

Cellulose is a polysaccharide containing a linear chain made up of several hundred to over ten thousand β (1 \rightarrow 4) linked D-glucose units. The cellulose derivatives used in topical ophthalmic formulations are methyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose (HPMC), sodium carboxymethyl cellulose (NaCMC) [34]. At low concentrations (1-10%), their aqueous solutions exist as a liquid but form gels upon heating. The high phase transition temperature exhibited by cellulose derivatives can be lowered by physical or chemical modification [35]. The transition temperature is between 40 and 50 °C for MC and between 75 and 90 °C for HPMC. Addition of sodium chloride is known to lower the gelation temperature of MC to 32-34 °C, while the transition temperature of HPMC can be decreased to about 40 °C by lowering the hydroxypropyl molar substitution [36].

Chitosan

Chitosan is an aminopolysaccharide (Fig. 5) made from the partial deacetylation and depolymerization of chitin, which is found in the exoskeletons of arthropods, such as crustaceans [37]. Commercially, chitin is mainly derived from the shell wastes of shrimp, crab, krill, lobster, and squid [26]. Chitosan has been proven to possess many advantages in biomedical applications due to its biocompatibility, biodegradability, mucoadhesiveness with low immunogenicity and low cytotoxicity [38]. Recently, chitosan-based thermosensitive gels with different polyols such as ethylene glycol, glycerol, and sorbitol have attained much popularity [39].

The derivatization of primary amino groups of chitosan (CS) by thiol groups results in the formation of Thiolated Chitosan (TCS). TCS based drug delivery system is gaining attention because it exhibits high mucoadhesive strength and extended drug release properties. TCS shows in-situ gelling properties because of the formation of intra and intermolecular disulfide bonds as a result of oxidation of thiol groups at physiological pH-values [40].



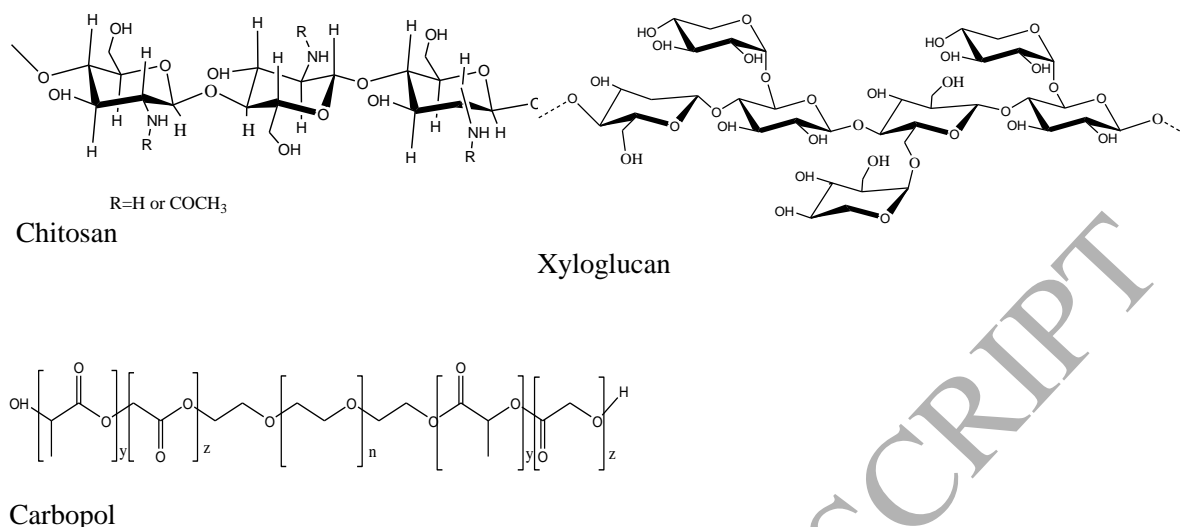


Fig. 5-The chemical structure of some in-situ gel polymers

3.2.1.2 Research progress in temperature triggered in-situ gel systems

Over the last decades, a large number of studies on temperature triggered in-situ forming system have been reported (Table 1). To mention some of them, Li et al. formulated Brinzolamide drug-resin in-situ thermosensitive gelling system, using Poloxamer F127 in combination with Carbopol 934P. The optimal formulation displayed a gel formation at 33.2 ± 1.1 °C and the diffusion-controlled release of the model drug over a period of 8 h. The *in vivo* study suggested that the in-situ gel demonstrated a better ability in retaining the drug than commercial formulations [41].

Khateb et al. also investigated the in-situ gelling system containing ofloxacin using a combination of Pluronic (PF-127 and PF-68) and sodium alginate. The incorporation of Pluronic F68 to Pluronic F127 solutions was found to rises the sol-gel temperature of binary formulation to above the physiological range of temperatures. The superior *in vitro* drug retention performance on glass surfaces and freshly excised bovine corn were exhibited by 20% (w/w) Pluronic F127 in comparison with other formulations. Additionally, *in vivo* evaluation in rabbits demonstrated that a retention performance of 20% (w/w) Pluronic F127 was higher than that of Pluronic F68. Furthermore, the slug mucosa irritation assay and bovine corneal erosion studies demonstrated no significant irritation was observed that resulted from these polymers and their combinations [42].

Osswald et al., prepared an injectable microsphere-hydrogel by loading the anti vascular endothelial growth factor, anti-VEGF (ranibizumab or aflibercept) into poly (lactic-co-glycolic acid) microspheres that were then suspended within an injectable poly(N-isopropylacrylamide)-based thermo-responsive hydrogel DDS. The efficacy was evaluated *in vivo* in a laser-induced rat model of choroidal neovascularization (CNV). CNV lesion area was measured and quantified by fluorescein angiograms and a multi-Otsu thresholding technique, respectively. Intraocular pressure (IOP) and dark-adapted electroretinogram (ERG) were also measured pre- and post-treatment (1, 2, 4, 8, and 12 weeks). The result has shown that the anti-VEGF-loaded DDS group had exhibited a significantly smaller CNV lesion areas than a non-treatment group of animals throughout the study, which suggests that the DDS offer a significant benefit in the management of posterior segment eye diseases [43].

The addition of cellulose derivates to Pluronic F12 hydrogels assist in increasing the bioavailability of the in-situ gel [44], Morsi et al., prepared Ketorolac tromethamine nanodispersions formulated into thermo-sensitive in-situ gel using Eudragit RL100. The study demonstrated that reducing the concentration of Pluronic F-127 was found to increase the gelation time and gelling temperature of the in-situ gels. The incorporation of HPMC to pluronic F12 hydrogels has significantly improved the mucoadhesive strength of the gel [45].

Addition of salts (NaCl and KCl) to in-situ gel system has known to decrease the gelation temperature. Bhowmik et al., examined the influence of different salts on the gelation properties, rheology and drug release of in-situ gel based on methylcellulose (MC). It was found that 5% -7% (w/v) sodium chloride, 8% - 9% (w/v) potassium chloride, or 5% (w/v) sodium bicarbonate was capable of reducing the GT below physiological temperature, i.e. 37 °C. The duration of drug release increased from 1.5 h to 3-5 h from salt containing MC solutions depending on the concentration and the type of salt [46]. Similarly, Bhowmick et al., confirmed that the use of i-carrageenan with potassium chloride could effectively decrease the GT of the virgin MC solution from 60 °C to 33.5 °C which is below physiological temperature [47].

The mixture of poloxamer with a mucoadhesive agent (chitosan) is known to extend the retention time of drugs for the treatment of ophthalmic diseases. Gratieri et al., formulated in-situ forming gel consisting the combination of poloxamer and chitosan. The results demonstrated that the addition of chitosan could improve the mechanical strength as well as texture properties of

poloxamer formulations and the in-situ gel increased a four-fold retention time in comparison with a conventional solution [48].

In addition to Poloxamer, Poly (N-isopropylacrylamide) (PN) has been widely used as thermo-responsive polymers. For instance, Hsiue et al., developed ophthalmic formulation using PN as the thermo-sensitive polymer. The clear solution of PN was known to form a gel upon the raising of temperature from the room temperature to about 32 °C. Epinephrine loaded linear PN and crosslinked PN nanoparticles were developed and evaluated. The finding of the study showed that the pressure decreasing the activity of the formulation with linear PN and combination of linear PN and crosslinked nanoparticles lasted six-fold and eight-fold longer than that of the conventional eye drop, respectively [49].

Recently, the studies have shown that copolymerization of PN with hyaluronic acid (HA) has increased the LCST of PN from 32 °C to above body temperature, which is more appropriate for the ophthalmic application. With this aim, Zhu et al., developed thermo-sensitive in-situ forming gelling formulation of Ketoconazole (KCL) based on PN/HA. The *in vitro* gelation, drug release, and antifungal activity were evaluated for the developed formulations. The gelation temperature of the PN-HA thermo-gelling solution was found 33 °C. The moderate release of KCL from in-situ gels without burst effects was exhibited. No macroscopic signs of irritation, redness, or other toxic effects were observed. The *in vivo* antimicrobial study indicated that KCL PN-HA in-situ gels displayed an improved cure percent as compared with commercial eye drops [3].

Very recently, Iohara et al., developed a hydrophobically modified hydroxypropyl methylcellulose (HM-HPMC) gel formed thermo-responsive hydrogels by incorporation of small amount of α -Cyclodextrin (α -CD) into the solution. The formed HM-HPMC/ α -CD gel exhibited a reversible sol-gel transition within the range of physiological temperature which was totally opposite to the temperature dependency has shown by the original HM-HPMC (without α -CD). The HM-HPMC/ α -CD exhibited the rapid gelation on the ocular surface and a significantly improved ocular drug (diclofenac sodium) absorption [50].

Table 1- Some examples of thermo-sensitive in-situ gelling system

Model drugs	Polymers	Major finding	Ref.
Brinzolamide	Poloxamer F127 and carbopol 934P	A sol-gel at $33.2 \pm 1.1^\circ\text{C}$ controlled release of drug over a period of 8 h.	[41]

Ofloxacin	Pluronic (PF-127 and PF-68) and sodium alginate	<i>In vivo</i> evaluation in rabbits exhibited improved retention performance of 20% (w/w) Pluronic F127 compared to Pluronic F68.	[42]
Ketorolac tromethamine	Pluronic F-127 HPMC K4M	Improved its ocular availability and prolonged its residence time.	[45]
Sparfloxacin	Pluronic (PF 127 and PF 68)	Showed promising antimicrobial activity <i>in vitro</i> and <i>in vivo</i> .	[51]
Fluconazole	Poloxamer/tween/carbopol	The <i>in vivo</i> ophthalmic absorption was superior to the conventional eye drop.	[52]
Lomefloxacin	Pluronic F127, Pluronic F68 and sodium alginate	Revealed a sustained release profile of 8 h.	[53]
Methazolamide	Poloxamer 407 and poloxamer P188	Had a better ability to retain drug than the eyedrops.	[54]
Diclofenac sodium	Pluronic F127	The bioavailability of diclofenac sodium in aqueous humor was significantly increased.	[55]
Dorzolamide hydrochloride	Poloxamer 407 and Poloxamer 188	Better pharmacological effect, faster onset of action, and prolonged effect relative to either drug solution or the market product.	[56]

3.2.2 pH triggered in-situ gelling systems

This in-situ gelling system consists of pH-sensitive polymers which are polyelectrolytes contain an acidic (carboxylic or sulfonic) or a basic group (ammonium salts) that either accept or release protons in response to alteration in pH in the surrounding environment. At lower pH (pH 4.4), the formulation exists as a regular solution, however, it undergoes gel formation at pH 7.4, that is the pH of tear fluid.

3.2.2.1 Polymers used in pH triggered in-situ gel systems

The most commonly used pH-responsive polymers in ophthalmic preparation are Polyacrylic acid (PAA, Carbopol 940), polycarbophil, and cellulose acetate phthalate (CAP) [17].

Carbopol (Polyacrylic acid)

Carbopol is a polyacrylic acid (PAA) polymer (Fig. 5), that displays a sol-gel phase transition in aqueous solution as a result of raising the pH above its pK of about 5.5 [57]. The carboxylic groups of PAA accept and release protons at low pH values and high pH values, respectively. Therefore, at high pH, the PAA swells due to the electrostatic repulsion of the negatively charged groups, releasing the drug molecules to the environment [17]. It is extensively exploited in ocular formulation with the aim of improving pre-corneal retention time of drugs. Carbopol provides

the benefit of exhibiting superior mucoadhesive properties as compared to other polymers. Mucoadhesive properties of carbopol is attributed to the interaction of poly(acrylic acid) with mucin that occurs by four mechanisms viz. electrostatic interaction, hydrogen bonding, hydrophobic interaction and inter diffusion [35]. Despite carbopol displays excellent mucoadhesive properties, the acidic nature of the gel is a major drawback which leads to irritation and damage to the eye tissues. Therefore, combinations of carbopol with other polymers including chitosan and HPMC were subsequently developed to overwhelmed this problem [25].

3.2.2.2 Research progress in pH triggered in-situ gel systems

The in-situ pH-triggered gelling system has a great potential to keep drug product more stable and retain drug release (Table 2). With this aim, our research group (Wu et al.) designed and evaluated pH-triggered gel containing Baicalin for sustained ophthalmic drug delivery using Carbopol 974P as the gelling agent along with HPMC E4M (0.6%, w/v) that was a viscosity enhancing agent. The *in vitro* and *in vivo* evaluations were conducted using confocal scanning light microscopy, rheometry, Gamma scintigraphic technique and microdialysis method. The result of rheological study displayed that the gel strength was significantly enhanced under physiological condition. The gel could provide sustained release of the drug over an 8 h period. Furthermore, the AUC and C_{max} values were found 6.1-times and 3.6-times higher than those of the control solution, respectively [58].

In addition, our research group (Pang et al.) have confirmed that the ocular in-situ gel can reduce the systemic absorption of the drug and thus reduce the potential systemic toxicity. Brimonidine Tartrate in-situ gel was prepared using Carbopol 974 P and HPMC E4M, and its therapeutic efficacy and systemic absorption were compared with that of eye drop. The pharmacodynamics study on the eye of rabbit showed that the gel formulation could significantly decrease intraocular pressure (IOP) as compared to the eye drop. More importantly, the *in vivo* pharmacokinetic studies exhibited that the plasma $AUC_{(0-\infty)}$ was found lower for the in-situ gel than the eye drop, which suggests the decreased systemic absorption [59].

Table 2- Some examples of pH-triggered in-situ gelling system

Model drugs	Polymers	Major finding	Ref.
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Baicalin	Carbopol 974P with HPMC E4M	Better stability, ocular bioavailability and sustaining drug release compared to commercial baicalin eye drops.	[58]
Ciprofloxacin	Calcium alginate with HPMC K4M and E50LV	Added benefits of sustained drug release.	[5]
Norfloxacin	Carbopol 934P.	Sufficiently mucoadhesive, antibacterial activity and free from ocular irritancy.	[60]
Timolol Maleate	Carbopol and chitosan	Showed a controlled type of release over 24 h periods.	[61]
Brimonidine	Carbopol 974 P and HPMC E4M	Increased efficacy and reduced systemic absorption of brimonidine tartrate.	[59]
Gatifloxacin	Carbopol 940 combined with HPMC and HPMC K15M	Provided sustained drug release over an 8-hour period.	[62]
Moxifloxacin	carbopol/HPMC	Showed increased in precorneal residence time, ocular bioavailability.	[63]

3.2.3 Ion-activated in-situ gel system

Ion-activated in-situ gelling systems form a crosslink with cations exists in the tear fluid (Na^+ , Ca^{2+} and Mg^{2+}), thus forming a gel on the ocular surface, which give rise to a extended corneal contact time (Table 3) [17, 64].

3.2.3.1 Polymers used in Ion-activated in-situ gel system

The most commonly used ion-activated polymers in ocular formulations are gellan gum (Gelrite[®]), hyaluronic acid and sodium alginates [65].

Gellan gum

Gellan gum are polysaccharides that can be used to induce ion-sensitive hydrogels. It is a linear anionic heteropolysaccharide (Fig. 5) made up of a tetrasaccharide repeating unit of glucose, glucuronic acid and rhamnose in the ratio of 2:1:1 [66]. Gellan comprises hydroxyl and carboxylic functional groups, which may interact with other polymers via hydrogen bonding and/or electrostatic attractions [67]. A low-acetyl gellan gum is commonly available in the market as Gelrite[®], which undergoes gelation in the presence of mono- or divalent cations. The electrolytes of the tear fluid especially Na^+ , Mg^{2+} and Ca^{2+} cations are particularly known to induce gel formation of the polymer upon instillation as a liquid solution into the cul-de-sac [68]. The incorporation of optimal quantities of calcium gluconate to gellan formulations lead to the formation of gellan calcium gluconate-simulated tear fluid (STF) gels with a significantly higher

strength than when gellan alone was mixed with STF [67]. It undergoes gelation by both temperature sensitive or cations induced mechanism. The possible mechanism of gelation includes the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by hydrogen bonding with water and complexation with cations [26].

Alginate/ Alginic acid

Alginate is a linear co-polysaccharide derived from brown seaweeds and some bacteria. Chemically it is a (1-4)-linked block copolymer of α -D-mannuronate (M) and its C-5 epimer R-L-guluronate (G), with residues arranged in homopolymeric sequences of both kinds and in region which approximate to the disaccharide repeating structure (MG) [69]. Sodium alginate undergoes gel formation as a result of calcium alginate formation by virtue of its interaction with a divalent cation (Ca^{2+}) present in lachrymal fluid (pH 7.4) [5]. Various properties of the polymer such as mechanical strength, porosity, etc. are highly depend on the ratio of β -D-mannuronic acid and α -L-glucuronic acid. Alginate with a high guluronic acid content exhibit a better gelling properties and minimize the concentration of polymer required to form stiff gel [26].

Pectin

Pectins are a polysaccharides family, where the polymer backbone mostly consists of α -(1,4)-D-galacturonic acid residues. Low methoxy pectins which are with a degree of esterification $< 50\%$ can form gel in aqueous solution in the existence of free calcium ions, that cross link the galacturonic acid chains. Its water solubility is one of the important advantages of pectin, therefore organic solvents can be avoided in the formulations [70]. The in-situ gelling of pectin induced by calcium ions exists in lacrimal fluid has been reported in a US patent. In addition, pectin based in-situ gel has been used to prolong drug release from the formulations such as theophylline, acetaminophen, and cimetidine [71].

3.2.3.2 Research progress in ion-activated in-situ gel systems

Various ion-activated in-situ gelling systems have previously been reported. Rupenthal et al., formulated ion-activated in-situ based on gellan gum, xanthan gum and carrageenan, and *in vivo* release, precorneal retention time and the ocular irritancy were characterized for the formulations. The results showed that the in-situ system was non-irritant with increased AUC and

the miotic response of pilocarpine by 2.5-fold compared to an aqueous solution [64].

Zhu et al., also developed an ion-activated ketotifen ocular formulations using a natural polysaccharide which is deacetylase gellan gum. The study demonstrated that deacetylase gellan gum had a potential to prolong the residence time of the formulation. The in-situ gels exhibited a characteristic sustained and extended drug effects behavior compared with the conventional eye drops at the same dose [72].

Kesarla et al., formulated nanoparticles loaded ophthalmic in-situ gel using the ion-sensitive polymer gellan gum used as a gelling agent which could form gel immediately and remained for the extended time of period. The developed formulation was found stable and displayed improved corneal contact time and minimizing the frequency of administration. The confocal microscopic study showed a clear cornea permeation of drug loaded nanoparticles [73]. Tayel et al., developed a novel ion-sensitive in-situ ophthalmic nanoemulsion (NE) gels containing terbinafine hydrochloride. The optimized in-situ NE gel exhibited a significantly higher C_{max} , delayed t_{max} , prolonged mean residence time and enhanced ocular bioavailability [74].

In the development of bioadhesive ion-sensitive hydrogels, the incorporation of the poorly water soluble drug is very challenging. Cyclodextrins (CDs) are beneficial pharmaceutical excipients that assist in the formulation of poorly aqueous soluble drugs. The inclusion of hydroxypropyl- β -cyclodextrin (HPBCD) in the in-situ formed gel has shown to allow a more effective control and a significant improvement in the fluconazole release [66].

Table 3- Some examples of ion-activated in-situ gelling system

Model drugs	Polymers	Major finding	Ref.
Gatifloxacin	Alginate with HPMC	A higher ocular bioavailability and extended residence time in aqueous humor than conventional ophthalmic solutions.	[75, 76]
Fluconazole	HPBCD complexed gellan gum and κ -carrageenan	Showed effective control of fluconazole release and good bioadhesive properties.	[66]
Acetazolamide	Gellan gum with xanthan gum, HPMC or carbopol.	Enhanced therapeutic efficacy and more extended intraocular pressure lowering effect compared to that of marketed eye drops and oral tablet.	[77]
Terbinafine hydrochloride	Gellan gum	Significantly higher C_{max} , delayed t_{max} , and prolonged mean residence time and increased bioavailability.	[74]

Antisense oligodeoxynucleotide	Gellan gum and carrageenan	The greatest reduction in wound size, the least stromal edema and hypercellularity	[78]
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3.2.4 Multi-stimuli responsive in-situ gel

One of the recent excellent strategies in ocular in-situ gelling system is the use of a combination of polymers with the different gelling mechanism, which have shown an improved therapeutic efficacy and better patient compliance [20]. Over last current years, a number of investigations that involved the combination of thermo-responsive polymers, pH-sensitive polymers or ion-activated polymers in the same ophthalmic formulation have been reported (Table 4).

Khan et al. developed and evaluated sparfloxacin loaded novel in-situ gelling system for sustained ophthalmic drug delivery using a combination of ion and pH activated gelling system, which were sodium alginate and methylcellulose, respectively. The formulation was in sol form at pH (4.7) and has undergone quick sol-gel transition upon raising pH to 7.4. The in-situ gel formulation demonstrated *in vitro* sustained release of sparfloxacin over a period of 24 h as compared to eye drop. The *ex vivo* corneal permeation study on goat eye revealed that a dramatically improved permeation as compared to eye drop [21].

In addition, Yu et al., reported nepafenac in-situ gel using carboxymethyl chitosan (CMC) and poloxamer composed of PEO-PPO-PEO block copolymer which was found to undergo a reversible sol-gel transition upon a change in a temperature and/or pH at a very low concentration. The result of a CCK-8 (Cell Counting Kit-8) study showed that the formulation was not toxic to human corneal epithelial cells at a low concentration. The formulation of poloxamer-CMC/NP showed a sustained release of nepafenac from the hydrogel. The release rate was found to be maximum at 35 °C and pH 7.4 [79].

Davaran et al., developed a dual thermo-/pH-responsive nanocarriers in-situ gel for ciprofloxacin. Ciprofloxacin released from the nanoparticles in in-situ gelling system demonstrated an improved antimicrobial activity as determined by minimal inhibitory concentrations [80]. Gupta et al., also formulated in-situ gel using the combination of gellan gum (ion-sensitive) and chitosan (pH sensitive) so as to improve precorneal residence time of sparfloxacin. Accordingly, the developed sparfloxacin in-situ forming gel was found nonirritant and showed the prolonged retention at the corneal site with the prolonged drug release [81].

Table 4- Some examples of multi-stimuli responsive in-situ gelling system

Model drugs	Polymers	Stimuli	Major finding	Ref.
Sparfloxacin	Sodium alginate and methylcellulose	Ion and pH sensitive	Rapid gelation upon raising pH to 7.4, <i>in vitro</i> sustained drug release over period of 24h, significantly enhanced corneal permeation.	[21]
Nepafenac	Carboxymethyl chitosan (CMC) and poloxamer	pH-induced and thermo-sensitive	The gelation temperature of 32-33 °C and retarding the drug diffusion rate was observed.	[79]
Timolol	Chitosan with gellan gum	pH-sensitive and ion-activated polymer	Enhanced transcorneal drug permeation and prolonged the retention at the corneal site.	[82]
Levofloxacin	Sodium alginate and chitosan	Ion and pH-triggered	Better retention time was observed.	[83]
Ciprofloxacin	Carbopol/HPMC and Poloxamer	pH-induced and thermo-sensitive	Empoved therapeutic efficacy and offers sustained release of the drug over an 8h period.	[84]

3.3 Nano-in-situ gelling systems

Nowadays nanotechnology is the most emerging concept in pharmaceutical sciences [85]. Several nano-technology based formulations have been developed to extend ocular residence time and to improve bioavailability of ophthalmic drugs. However, nanoparticles have not mucoadhesive property, so are cleared out of eyes rapidly [86]. The suspending of fabricated nanoparticles in an in-situ gelling vehicle which undergoes sol to gel phase transition upon exposure to physiological condition is known to solve this problem. The nanoparticulate in-situ gel was designed to explore the double benefit of nanoparticles and in-situ gelling system, for its ophthalmic delivery (Table 5) [60, 87]. This results in extending the pre-corneal residence time of the nanoparticles and improving ocular bioavailability [26].

The formulations of in-situ gel in novel drug delivery system as colloidal carriers systems such as nanosuspensions, lipid-based nanocarriers, have proven to be the most effective strategy, causing an exponential increase in the bioavailability of the ophthalmic drugs. For instance, Liu et al., developed the curcumin (CUR) loaded ocular nanogel by using cationic nanostructured lipid carriers (CNLC) and thermosensitive gelling agent. The *in vitro* release, corneal permeation, ocular irritation and preocular retention were evaluated. Also, the pharmacokinetic

profile in the aqueous humor was evaluated by microdialysis technique. The AUC of the nanogel (CUR-CNLC-GEL) was found 9.24-times higher than those of curcumin solution (CUR-SOL), indicating a significantly improved bioavailability [88].

Pandurangan et al., formulated solid lipid nanoparticles (SLNs)-loaded in-situ gel with voriconazole which was found to be a promising vehicle for ocular delivery with good stability and excellent zone of inhibition in the microbial assay of voriconazole [89]. Paradkar et al., developed a niosomal in-situ gel using Poloxamer 407 and HPMC K4M. The prepared Natamycin niosomal in-situ gel formulation exhibited an increased corneal retention time due to bioadhesive property of gel and displayed extended drug release up to 24h which as compared to marketed products. The formulation was also found to exhibit a better transcorneal permeation [90]. Shukr et al., also formulated voriconazole loaded in-situ gelling niosome for ocular inserts using span 40 and span 60 with pluronic F127 and pluronic L64. The optimized in-situ gelling ocular insert showed a significantly higher C_{max} , delayed t_{max} and increased bioavailability, and was found non-irritant [91].

Microsphere-loaded ion-activated in-situ gel of ofloxacin (OFX) was also formulated. *In vivo* results in rabbits exhibited that OFX-loaded microspheres in-situ gel could improve the relative bioavailability by 11.7-times relative to the commercial OFX eye drops. Furthermore, the extended duration of action of OFX-loaded microspheres in-situ gel preparation is thought to avoid frequent administration, which improves patient compliance [92].

Nofloxacin loaded nanocarriers were designed utilizing chitosan as a matrix forming polymer, cross-linked by an anionic cross linker sodium tripolyphosphate. The developed chitosan nanoparticulate in-situ gel exhibited superior performance over the marketed eye drops [60]. Levofloxacin nanoparticles laden in-situ gel was formulated and showed the improved ocular retention time. Gupta et al., designed nanoparticle laden in-situ gel encapsulated PLGA nanoparticle, containing levofloxacin, added into chitosan in-situ gel. Ocular retention was evaluated by gamma scintigraphy in rabbits. The developed nanoparticle laden in-situ gel formulation exhibited slow rate of clearance and retained at the corneal surface for more extended duration than commercially available formulation, in-situ gel and nanosuspension alone [93]. Furthermore, the same group of research confirmed for excellent sustained release of the nanoparticle in-situ gelling system containing sparfloxacin [94].

More recently, Ahmed et al., formulated ketoconazole poly(lactide-co-glycolide) nanoparticles with subsequent loading into in-situ forming gel for ophthalmic drug delivery system. The *in vitro* release of the drug from the formulations loaded with nanoparticles displayed sustained and greater drug release compared to free drug formulations. In addition, the in-situ gelling with nanoparticles showed improved anti-fungal activity in comparison to pure drug formulations. Alginate-chitosan in-situ gel containing optimized ketoconazole nanoparticles displayed higher drug permeation via epithelial cell lines [95].

Table 5- Some examples of nanocarrier in-situ gelling system

Model drugs	Polymers	Type of stimuli-nanocarrier	Major finding	Ref.
Timolol	Gellan gum	Ion-triggered-liposome	Rapid reduction of intraocular pressure and significantly longer effective time.	[96]
Dorzolamide	Poloxamer 407	Thermo-sensitive-nanoemulsion	Non-irritant and highly therapeutically efficient.	[56]
Loteprednol	Poloxamer 407 and 188	Thermo-sensitive-nanoemulsion	Extended mean residence time and improved (2.54-times) bioavailability compared to marketed formulation.	[28]
Cyclosporine A	Deacylated gellan gum	Ion-triggered-microemulsion	Showed 3-fold greater bioavailability.	[97]
Acetazolamide	Carbopol 934	pH-triggered-nanoparticles	Higher permeation, longer precorneal residence time and sustained release of the drug along with improved <i>in vitro</i> efficacy.	[98]
Acetazolamide	Gellan gum, xanthan gum, HPMC/carbopol	Ion-triggered-nanoemulsion	Showed higher therapeutic efficacy and more prolonged intraocular pressure lowering effect relative to that of commercial eye drops and oral tablet.	[77]
Ketorolac	Pluronic®F-127, HPMC K4M	Thermo-sensitive-nanodispersion	Sustained the release of drug, improved its ocular availability and prolonged its residence time without causing irritation to eye.	[45]
Curcumin	Poloxamer 188 and 407	Thermo-sensitive-nanostructured lipid carriers	Significantly enhanced precorneal retention and ocular permeation capacity.	[88]

4 Clinical application of in-situ gelling

To date, some of in-situ gel formulations have been commercially available for ocular drug delivery (Table 6). For instance, Timoptic-XE[®], containing timolol maleate (0.25% and 0.5%) in gellan gum has been available on market since 1994, which is applied topically on the eye to

treat glaucoma. Furthermore, some of the patents on in-situ gel for ocular delivery system have been issued in the last decades, and are being summarized in Table 7.

Table 6 - List ocular in-situ gels approved for market

Name of the product	Polymer	The type of in-situ gelling system	Company	Ref.
Timoptic-XE® (Timolol maleate ophthalmic gel forming solution)	Gellan gum	Ion-induced	Merck Pharmaceuticals, USA	[99]
Pilopine HS® (pilocarpine hydrochloride ophthalmic gel)	Carbopol 940	pH-triggered	Alcon laboratories, inc. USA	[26]
Akten® (Lidocaine hydrochloride)	HPMC	Temperature-triggered	Akorn Inc., Lake Forest, IL	[100]
AzaSite (azithromycin ophthalmic solution)	Poloxamer 407	Temperature-triggered	InSite Vision	[101]
Timoptol-LA (Timolol maleate)	Gellan gum	Ion-activated	Laboratories Merck Sharp and Dohme	[25]
Virgan (Ganciclovir)	Carbopol® 974	pH-triggered	Laboratoires THEA-France	[25]

Table 7 - List of some patents of in-situ gelling system for ocular delivery

Patent Number	Title of the patent	Gelling agents	Year of publication	Ref.
US 2011/0082128 A1	In-situ gel ophthalmic drug delivery system of estradiol or other estrogen for prevention of cataracts	Deacetylated gellan gum	2011	[102]
US 2002/0114778 A1	Reversible gelling system for ocular drug delivery	A block copolymer of propylene oxide and ethylene oxide with HPMC	2002	[103]
US 8,343,471 B2	Nanoparticulate in-situ gels of TPGS, gellan and PVA as vitreous humor substitutes	Gellan with PVA	2013	[104]
WO 2011018800 A3	In-situ gel forming solution for ocular drug delivery	A combination of natural polysaccharide, thermoreversible polymer	2011	[105]
US 6,703,039 B2	Reversible gelling system for ocular drug delivery	A block copolymer of propylene oxide and ethylene oxide with HPMC	2004	[106]
US 6,511,660 B1	Ophthalmic drug delivery formulations and method for preparing the same	Carbopol and Pluronic	2003	[107]

5. Conclusions and future prospects

Despite the challenges in ocular drug delivery, over the past few years, many innovative approaches are being developed to overcome the problems associated with conventional of ophthalmic preparations. The in-situ gelling system is one the promising and extensively studied strategies that could prolong precorneal resident time and offer the sustained release drug delivery, thus improve ocular bioavailability and therapeutic efficacy and reduce systemic absorption and toxicity. Furthermore, due to its drug release sustaining ability and decrease the frequency of administration, in-situ gel could improve patient compliance. In in-situ gel formulation with different stimuli-responsive polymers that have high sensitivity to change in pH, temperature, and ion concentration are used. However, the combination of two or more stimuli-responsive polymers in the same formulation is known to exhibit greater compliance and improved therapeutic efficacy. Moreover, exploring the combination of different drug delivery approaches (i.e. nanoparticles loaded in-situ gelling) to develop in-situ gel has been the attractive strategies to improve ocular drug delivery system.

As the eye is the most essential and sensitive part of the body, the safety issues of ophthalmic formulations is critically important. The majorities of the cytotoxicity and irritability studies included in this review showed that no significant alterations or sign of toxicity due to the application of in-situ gel. However, further studies are required to evaluate the possible toxicity due to repeated and long term applications and materials for the preparation of nanoparticles in nano-gel systems. In addition, the increased viscosity in in-situ gel may cause some limitations like blurred vision and discomfort to patient resulting in a faster elimination due to reflex tears and blinks. Therefore, critical control of the viscosity should be taken into consideration during designing and optimization of in-situ gel formulation in order to reduce the limitations to the tolerable level.

Despite the promising potential of in-situ gel in ocular drug delivery, only a limited number of drugs in the form of in-situ gel are currently in clinical use. Consequently, further works should be done to explore this drug delivery system for the clinical application of other ophthalmic drugs.

At present, most of the ophthalmic in-situ gels were designed only for the formulations containing of single active ingredient. In the future, some more suitable strategies should be developed for the formula consisting of multiple ingredients such as Traditional Chinese Medicine in particular, which involves a multi-target approach to produce their action. Lastly, in the future, we expect the innovation of new and more reliable in-situ forming polymers which may be responsive to some biochemical markers associated with the disease conditions of the eye.

Conflict of Interest

The authors affirm and confirm that there is no any conflict of interest issues with regards to the content of this article.

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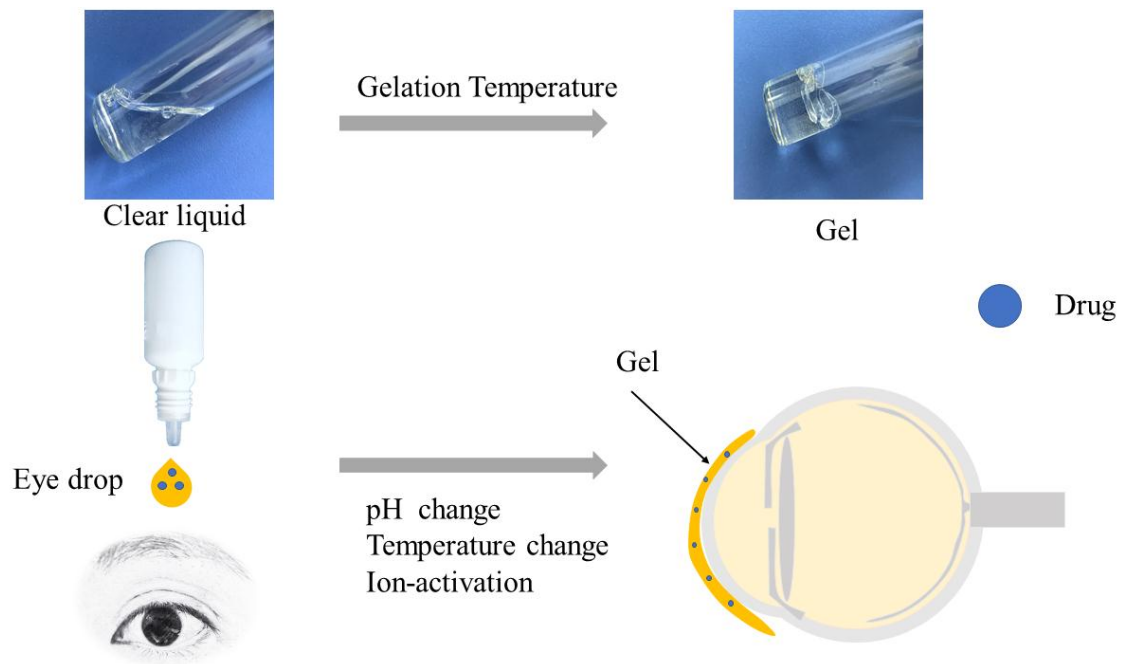
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Graphical abstract



ACCEPTED MANUSCRIPT